Pancreatic Hormones and Hepatic Methionine Adenosyltransferase in the Rat* (34029)

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(Introduced by H. Tarver)

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Methionine and adenosyltransferase (ATP: L-methionine S-adenosyltransferase, EC 2.5. 1.6) is the specific enzyme responsible for the formation of *S*-adenosylmethionine, a major donor of methyl groups in mammalian tissues (1). It plays a role in the regulation of the metabolism of single carbon units (2) and other intermediates (3).

Our previous studies (4–6) showed that the transferase activity in rat liver was significantly influenced by such nutritional and hormonal factors as adrenal glucocorticoids and dietary protein, indicating a close association with carbohydrate and protein metabolism. The actions of hormones and diets are known to be interrelated (7–10). However, the effects of hormones upon enzymes are not always predictable from their similar or antagonistic properties (11–15).

Since the pancreatic hormones, insulin (16) and glucagon (17, 18) have profound effects on carbohydrate and protein metabolism, we investigated their roles in the regulation of hepatic methionine adenosyltransferase in rats, the liver being the organ with a high concentration of the enzyme (19). The results indicate that insulin deficiency brought about a marked increase in the hepatic transferase activity by an adrenal-mediated process which appears to require a continued synthesis of the enzyme protein, whereas administration of excess glucagon has little effect on this enzyme. While the present work was in progress, an exploratory study of the effects of alloxan and glucagon administration on the transferase activity was reported (20).

Experimental Procedures. Animals. The materials and methods are essentially as outlined in previous papers (5, 6). About 2-month-old male and female Sprague-Dawley rats, weighing 130–180 g, were used. The animals were maintained on a commercial diet (Taiwan Sugar Corp.) and tap water or 1% NaCl *ad libitum* until the time of sacrifice unless otherwise specified. Bilateral adrenalectomies were performed under light ether anesthesia. Diabetes was induced by a single injection of 14 mg of alloxan monohydrate/100 g of body weight after fasting overnight and the rats were used if the blood glucose concentration was in excess of 300 mg/100 ml.

Assays. Rats were sacrificed by decapitation and blood was collected in heparinized beakers. Methionine adenosyltransferase was assayed, essentially according to Cantoni and Durell as described (6), in individual livers, freshly excised. Protein concentrations were measured by the biuret method (21) using crystalline bovine serum albumin as standard. Blood glucose was determined by the method of Hyverinän and Nikkilä as modified by Pryce (22).

Expression of results. Methionine adenosyltransferase activity is expressed in micromoles of *S*-adenosylmethionine formed per 30 min at 37° per liver per 100 g of body weight (total activity) or per mg of liver protein (specific activity). The data are presented as percentages of values found in the appropriate control animals (6) and given as means ± SE. The results were statistically evaluated by *t* test and no significant difference is indicated when the p value was >0.05.

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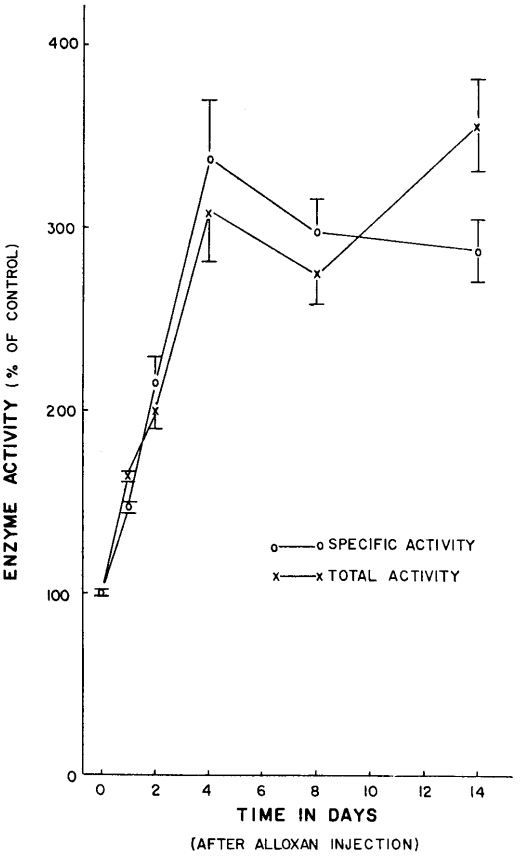

FIG. 1. Time course of the induction of hepatic methionine adenosyltransferase in alloxan-diabetic male rats.

Materials. Actinomycin D and 9α-fluoro-16α-hydroxy-1-dehydrocortisol (triamcinolone) were gifts from Merck, Sharp and Dohme and American Cyanamid Co., respectively. Insulin was purchased from Boots Pure Drug Co.; alloxan from Eastman Chemical Co.; glucagon from Sigma Chemical Co.; and cycloheximide, Salt Mixture USP XIV, and Vitamin Fortification Mixture from Nutritional Biochemicals Corp. All other chemicals used were of reagent grade.

Results. Effect of alloxan treatment. The time course of changes in hepatic methionine adenosyltransferase levels of alloxan diabetic male rats is shown in Fig. 1. The administration of alloxan caused a significant increase in the enzyme level within the first day with respect to both total amount of enzyme produced (per liver per 100 g of body wt) and activity per milligram of liver protein. Activity was up to 3–3.5 times above the untreated (zero time) level 4 days after the alloxan treatment. Then it appeared to stay at a new plateau. The longest period of observation was 14 days.

Effect of insulin administration to alloxan-diabetic rats. As presented in Fig. 2, both in the males and females, daily administration of insulin almost completely blocked the elevation of enzyme activity occurring in the untreated alloxan diabetic rats. Furthermore, insulin was also able to bring down the elevated enzyme level toward the normal range when it was given 3 or 4 days after alloxan. Thus it is clear that insulin deficiency causes an enhancement of the transferase activity in rat liver.

Involvement of the adrenals. There are two

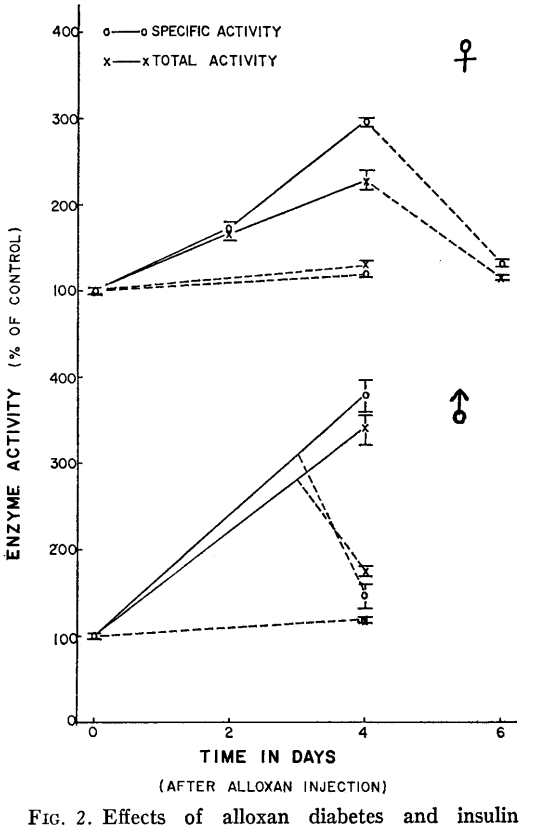

FIG. 2. Effects of alloxan diabetes and insulin treatment on the hepatic levels of methionine adenosyltransferase: diabetic rats were untreated (—) or treated (- - - -) with protamine zinc insulin intraperitoneally, 4 units/100 g/day.

TABLE I. Effect of Adrenalectomy on Response of Hepatic Methionine Adenosyltransferase to Alloxan-Diabetes in Female Rats.

Treatment	No. of rats	Enzyme activity (% of control)		Blood sugar (mg/100 ml)
		Sp act	Total act	
Control	4	100 ± 4.3	100 ± 4.6	106 ± 1.4
4 days after alloxan	4	209 ± 34.4[a]	221 ± 37.6[a]	539 ± 60.2
10 days after adX[b]				
Without alloxan	10	85 ± 5.9	87 ± 8.6	109 ± 1.5
4 days after alloxan	12	95 ± 3.5	93 ± 6.3	404 ± 22.3
8 days after alloxan				
Without adX	3	258 ± 39.1[a]	275 ± 71.3[a]	395 ± 55.3
4 days after adX	5	129 ± 8.3	113 ± 7.4	267 ± 66.2

[a] $p < 0.05$ as compared with the controls.
[b] adX = adrenalectomy.

possibilities as to the mechanism which gives rise to a marked increase of methionine adenosyltransferase in the livers of insulin deficient rats. One is that the level of the transferase in normal rat liver might be suppressed by insulin without involvement of any other hormones and the other is that some hormone(s) other than insulin might cause an elevation of the enzyme activity in the absence of insulin. Our experimental results presented in Table I show that alloxan treatment caused little change in the transferase activity in the livers of adrenalectomized rats. In addition, adrenalectomy restored the greatly increased enzyme activity in diabetic rats almost to the normal level. These results are in line with our previous findings that glucocorticoid treatment increased the transferase activity in rat liver (4, 5).

Combined effects of alloxan and glucocorticoids. Figures 3 and 4 show that at least an additive effect on the hepatic methionine adenosyltransferase level occurred when both alloxan and triamcinolone, a synthetic glucocorticoid, were administered simultaneously to male or female rats, while insulin was able to block the increase in the enzyme activity of the livers of alloxan diabetic rats brought about by treatment with triamcinolone.

Effects of cycloheximide and actinomycin D. The data shown in Tables II and III show that, under several experimental condi-

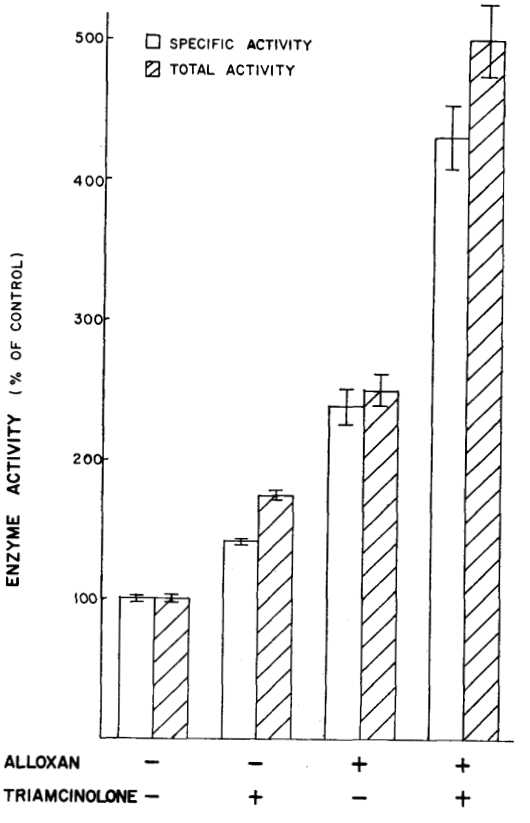

FIG. 3. Effect of alloxan diabetes on the induction of hepatic methionine adenosyltransferase by triamcinolone in male rats: 3 days after alloxan treatment, rats were given intraperitoneally a single dose (6 mg/100 g) of triamcinolone, suspended in a mixture of propylene glycol and normal saline (1:1, v/v). They were sacrificed 24 hr later.

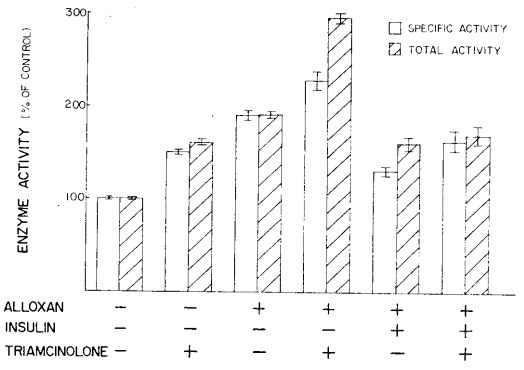

FIG. 4. Effect of insulin on the induction of hepatic methionine adenosyltransferase by triamcinolone in alloxan-diabetic female rats: the dosage for triamcinolone was the same as that shown in Fig. 3. Four units of protamine zinc insulin were injected intraperitoneally a few minutes before triamcinolone.

tions, the enhancement of methionine adenosyltransferase in the livers of alloxan-treated rats was prevented by administration of either cycloheximide or actinomycin D, which are inhibitors of protein (23) or RNA (24) synthesis, respectively. These results suggest that the synthesis of new protein and RNA is associated with the response of this transferase to alloxan diabetes.

Effect of blood sugar level. The possibility that the increase in hepatic methionine adenosyltransferase activity in alloxan diabetic rats is due to the elevated blood sugar level was studied. Tables I and II show that, although neither adrenalectomy nor the administration of cycloheximide blocked the rise of blood sugar levels, both treatments inhibited the increase in the hepatic methionine adenosyltransferase activity. The conclusion that there is no direct relationship between the enzyme activity and blood sugar levels was further supported by the glucose-feeding experiment. As shown in Table IV, starvation of rats previously fed a protein-free diet lowered the blood sugar level but slightly raised the enzyme activity. On the other hand, glucose feeding raised the blood sugar level, but not the enzyme activity, compared with those of the fasting rats. However, the increase in the transferase activity in the rats force-fed casein hydrolysate was partially prevented by simultaneous feeding of glucose (unpublished data).

Effect of the administration of insulin to normal rats. Since the elevated transferase levels in the livers of alloxan diabetic rats

TABLE II. Effect of Cycloheximide on the Induction of Hepatic Methionine Adenosyltransferase in Alloxan-Diabetic Rats.[a]

Expt. no.	Treatment	No. of rats	Enzyme activity (% of control) Sp act	Enzyme activity (% of control) Total act	Blood sugar (mg/100 ml)
1	Control	4	100 ± 4.1	100 ± 3.6	108 ± 1.5
	Alloxan (A)	5	132 ± 5.2[b]	125 ± 8.9	539 ± 71.2
	Cycloheximide (C)	5	120 ± 6.3	97 ± 3.9	97 ± 1.2
	A + C	5	112 ± 8.1	92 ± 5.5[c]	504 ± 5.5
	[Inhibition by cycloheximide (%)][d]		63	100	
2	Control	4	100 ± 5.7	100 ± 4.2	109 ± 1.7
	Alloxan (A)	3	173 ±14.3[b]	171 ±17.2[b]	360 ± 38.4
	Cycloheximide (C)	5	97 ± 3.0	78 ± 4.1	110 ± 1.8
	A + C	3	87 ± 1.9[c]	69 ± 2.9[c]	131 ± 14.3
	[Inhibition by cycloheximide (%)][d]		100	100	

[a] Female rats were given a single dose of alloxan at 0 hr. In Expt. 1, cycloheximide (50 μg /100 g) was injected at 0 and 12 hr and rats were sacrificed at 24 hr. In Expt. 2, cycloheximide was given at 0, 12, 24, and 36 hr, and rats were sacrificed at 48 hr. All injections were given intraperitoneally.

[b] $p < 0.02$ as compared with the controls.

[c] $p < 0.01$ as compared with the alloxan-diabetic group.

[d] The enzyme levels in control animals were subtracted in calculating these values.

TABLE III. Effect of Actinomycin D on the Induction of Hepatic Methionine Adenosyltransferase in Alloxan-Diabetic Rats.[a]

Treatment	No. of rats	Enzyme activity (% of control)	
		Sp act	Total act
Control	6	100 ± 4.3	100 ± 3.6
Alloxan (A)	9	139 ± 4.0[b]	133 ± 4.5[b]
Actinomycin D (A′)	5	102 ± 4.2	83 ± 4.2
A + A′	9	115 ± 5.5	91 ± 4.6
[Inhibition by actinomycin D (%)][c]		62	100

[a] Female rats were given intraperitoneally a single dose of alloxan 24 hr before sacrifice. Actinomycin D (50 μg/100 g) was injected intraperitoneally at 12 and 24 hr before sacrifice.

[b] $p < 0.05$ as compared with the values of both control and A + A′ groups.

[c] The enzyme levels in control animals were subtracted in calculating these values.

were reversed by insulin, it might be expected that administration of exogenous insulin to the nondiabetic rats would lower the enzyme activity below the normal. However, it was found that administration of insulin to intact rats for various periods of time always tended to increase, rather than decrease, the basal level of the transferase. The results are presented in Table V.

Effect of glucagon. Glucagon and insulin are antagonistic with respect to their effects on protein catabolism as judged by the rate of urea production (25). It has also been suggested that the effects of insulin deficiency on rat liver gluconeogenesis are a manifestation of the effects produced by endogenous glucagon and other lipolytic hormones (26). Therefore, the possibility that the elevation of hepatic methionine adenosyltransferase level in alloxan-diabetic rats is mediated by glucagon was examined. Table VI indicates that administration of glucagon to normal fed rats caused little change in the hepatic transferase level. Similar results obtained with rats fed a low-protein diet have been reported (20).

Discussion. The present studies show that the marked elevation of hepatic methionine adenosyltransferase level in alloxan diabetic rats is primarily, if not entirely, due to an insulin deficiency. Glucocorticoids and insulin-deficiency seem to act additively to enhance the induction of the transferase. The findings of this and previous (4, 5) studies are in accord with the coordinating roles of glucocorticoids and insulin in promoting and inhibiting gluconeogenesis. The effect of insulin on the hepatic level of methionine adenosyltransferase, however, would not be simply predictable from the known effect of glucocorticoids (4, 5) and the generally recognized insulin–glucocorticoid antagonism (27). For example, insulin was found to stimulate the synthesis of the glucocorticoid-inducible tyrosine transferase (12) and tryp-

TABLE IV. Effect of Blood Sugar Level on Hepatic Methionine Adenosyltransferase Activity.[a]

Treatment	Enzyme activity (% of control)		Blood sugar (mg/100 ml)
	Sp act	Total act	
Control	100 ± 2.8	100 ± 3.8	88 ± 2.8
Fasting, 1 day	124 ± 4.9[b]	126 ± 8.7[b]	65 ± 7.8
2 days	128 ± 5.2[b]	118 ± 5.8[b]	57 ± 4.7
Fasting, 1 day + glucose	103 ± 4.4	110 ± 9.4	358 ±92.0

[a] Four groups of female rats were fed with a protein-free diet (starch 83.8%; corn oil 10.0%; salt mixture, USP XIV, 4.0%; vitamin fortification mixture 2.2%) for a week. Eight rats were used as control; four each were fasted for 1 or 2 days, respectively; and the fourth group (9 rats) were fasted for 2 days but given glucose during the last 24 hr. Glucose was administered by gastric intubation, 1 g in 3 ml of water per 100 g of body weight at 24, 18, 12, and 1.5 hr before sacrifice.

[b] $p < 0.05$ as compared with the control values.

TABLE V. Effect of Insulin Administration on the Hepatic Methionine Adenosyltransferase Activity in Normal Rats.[a]

Insulin treatment (days)	Enzyme activity (% of control)	
	Sp act	Total act
Control	100 ± 5.6	100 ± 4.7
1	115 ± 6.3	115 ± 5.7[b]
2	117 ± 9.5	114 ± 6.9
3	128 ± 8.4[b]	128 ± 3.5[b]
5	131 ± 3.8[b]	130 ± 1.6[b]

[a] Female rats were used. In the first experiment, 2 groups of 6 rats were injected intraperitoneally with 2 units of regular insulin every 12 hr for 1 or 2 days; in the second experiment, 2 groups of 5 rats received 6 or 4 units of protamine zinc insulin intramuscularly daily for 3 or 5 days, respectively; control rats (4 for each experiment) received normal saline instead of insulin. All doses were per 100 g of body weight.

[b] $p < 0.05$ as compared with the control values.

tophan oxygenase (13) of rat liver. Insulin, as well as glucocorticoids, was found to increase the phenylethanolamine-N-methyl-transferase of rat adrenal (14). On the other hand, the apparent cortisol activation of hepatic glycogen synthetase was dependent on insulin (15).

Alloxan diabetes is known to cause hypertrophy and hyperfunctioning of the adrenal cortex of rats (28). Our results show that the response of hepatic methionine adenosyltransferase to alloxan treatment is prevented or reversed by adrenalectomy, indicating that the effect of alloxan diabetes on this enzyme is to a great extent mediated by the adrenal glands. The same has been found to be true of glutamate dehydrogenase (29), glucose 6-phosphatase and phosphoenolpyruvate carboxykinase (11). The effects of insulin and glucagon as inducers of rat liver tyrosine aminotransferase, however, are not dependent on adrenals (12).

The evidence that the effect of alloxan diabetes on the hepatic methionine adenosyltransferase is independent of hyperglycemia has been presented. A similar conclusion was drawn in the case of glucose 6-phosphatase and fructose 1, 6-diphosphatase (30). A separation of hypoglycemia and insulin induction of enzymes has also been demonstrated (14).

The present studies with actinomycin D and cycloheximide indicate that *de novo* synthesis of RNA and protein is responsible for the rise in the hepatic methionine adenosyltransferase activity brought about by alloxan diabetes. This is consistent with and further supports the previous suggestion (5) that the increase of the enzyme in the glucocorticoid-treated rats probably requires the new synthesis of RNA and protein, since the present studies also indicate that the effect of alloxan diabetes on this enzyme is mediated by the adrenal glands. However, further investigations are required to show whether this is due to an increased rate of synthesis or diminished rate of degradation of the enzyme, or both.

A slight but consistent increase in the transferase activity was observed in intact rats treated with insulin. The mechanism underlying this effect of insulin remains unclear. The present data (Table VI), and those of Finkelstein (20), seem to rule out the possibility that the increase in the hepatic transferase in alloxan diabetic rats is mediated by glucagon. In view of these findings, it would also appear unlikely that the apparently paradoxical effect of excess exogenous insulin on the transferase of intact rats could be due to the possible contamination of insulin preparations with glucagon. Insulin, as well as cortisol, is known to stimulate the uptake of α-aminoisobutyric acid-1-^{14}C into the isolated, perfused rat liver

TABLE VI. Effect of Glucagon on Hepatic Methionine Adenosyltransferase Activity.

Treatment	Enzyme activity (% of control)	
	Sp act	Total act
Control	100 ± 2.9	100 ± 2.9
Glucagon	87 ± 3.8[b]	92 ± 4.3

[a] Glucagon (1 mg/ml in 0.05 *M* glycine buffer, pH 9.0) was given intraperitoneally to female rats at the rate of 0.2 mg/100 g for each injection at 24, 18, and 12 hr before sacrifice. Each group had 8 rats.

[b] $p < 0.02$ as compared with the control value.

(31). Hager and Kenney (12) and Schor and Frieden (13) have reported an apparently paradoxical increase in insulin-inducible tyrosine aminotransferase and tryptophan oxygenase, respectively, in diabetic adrenalectomized rats.

Differences in response to the administration of glucagon have been reported for tyrosine aminotransferase and tryptophan oxygenase. Both were induced by cortisol, but only the former was induced by glucagon (32). In this respect, methionine adenosyltransferase resembles tryptophan oxygenase rather than tyrosine adminotransferase.

Summary. Our previous finding that hepatic methionine adenosyltransferase varies directly with the level of glucocorticoids prompted the investigation of this enzyme in the catabolic conditions associated with insulin deficiency and during the administration of excess glucagon. While glucagon administration had little effect, alloxan diabetes caused a marked elevation of the transferase level in rat liver. Combined treatment with alloxan and a glucocorticoid showed an additive effect. The influence of alloxan diabetes on this enzyme was independent of hyperglycemia. The response to alloxan diabetes could be prevented and reversed by either insulin administration or adrenalectomy. The effect of glucocorticoids on enhancing methionine adenosyltransferase activity in diabetic rats was also prevented by insulin. However, the administration of insulin to normal rats caused a slight but consistent increase, rather than decrease, in the basal level of the enzyme. The increase in the transferase activity in the livers of alloxan-treated rats was largely blocked by actinomycin D or cycloheximide, indicating the synthesis of new RNA and protein is associated with the response of this enzyme to alloxan diabetes.

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